Available online at www.sciencedirect.com
SCIENCE @ DIRECT®

ELSEVIER

Carbohydrate Research 338 (2003) 819–826

CARBOHYDRATE
RESEARCH

www.elsevier.com/locate/carres

The reaction of acetylacetone with amino sugars: implications for the formation of glycosylpyrazole derivatives

Warren C. Kett*

Department of Chemistry, Division of Environmental and Life Sciences, Macquarie University, North Ryde, NSW 2109, Australia

Received 15 October 2002; accepted 20 January 2003

Abstract

Glycosylpyrazoles are efficiently formed by reaction of saccharide hydrazones with pentan-2,4-dione (acetylacetone), but in aqueous buffer, pyrazole derivatives of amino sugars couple with a further equivalent of acetylacetone affording high yields of ketoenamines. These ketoenamines were considerably more stable than the ketoenamines formed from 2-amino-2-deoxy aldoses that have been described as intermediates in the classical Elson–Morgan reaction. Moreover, high yields of *per*ketoenamine derivatives were achieved with oligosaccharides derived from hydrolysis of chitosan. The removal of the ketoenamine moieties to regenerate the free amine was readily accomplished with aqueous hydrazine.

Keywords: Sugar hydrazone; Pyrazole; Ketoenamine; Chitosan; Carbohydrate labelling; Acetylacetone

1. Introduction

Pyrazole derivatives of sugars have been proposed for use in glycan analysis.[1] Acetylacetone was found to react smoothly and efficiently with saccharide hydrazones (Scheme 1),[1] but it also reacts with amines, complicating its use for the derivatisation of amino sugars. We have reexamined the reaction of acetylacetone with amino sugars under the conditions proposed for converting glycosylhydrazines to their pyrazole derivatives and have investigated both the possibility of exploiting the enamines formed and ways of removing them.

Successful handling of amino sugars is essential if the simple formation of pyrazoles is to offer an alternative to the established derivatisation procedures based upon reductive amination.[2,3] It would be especially useful when the glycans are converted to hydrazones by the cleavage procedure, such as hydrazinolysis,[4,5] or beta-elimination in the presence of hydrazine.[6]

The reactions of amino sugars with β-dicarbonyl compounds are well documented,[7–9] principally because 2,4-pentanedione is used in the Elson–Morgan procedure for the determination of 2-amino-2-deoxy aldoses. In fact, interest in the subsequent rearrangement of the ketoenamines to the Elson–Morgan products has tended to overshadow the investigation of the ketoenamines themselves, and our reexamination was prompted by the finding that the properties of ketoenamines were not entirely as expected.

2. Results

2.1. Formation and stability of the ketoenamine from 1-glucosaminylpyrazole

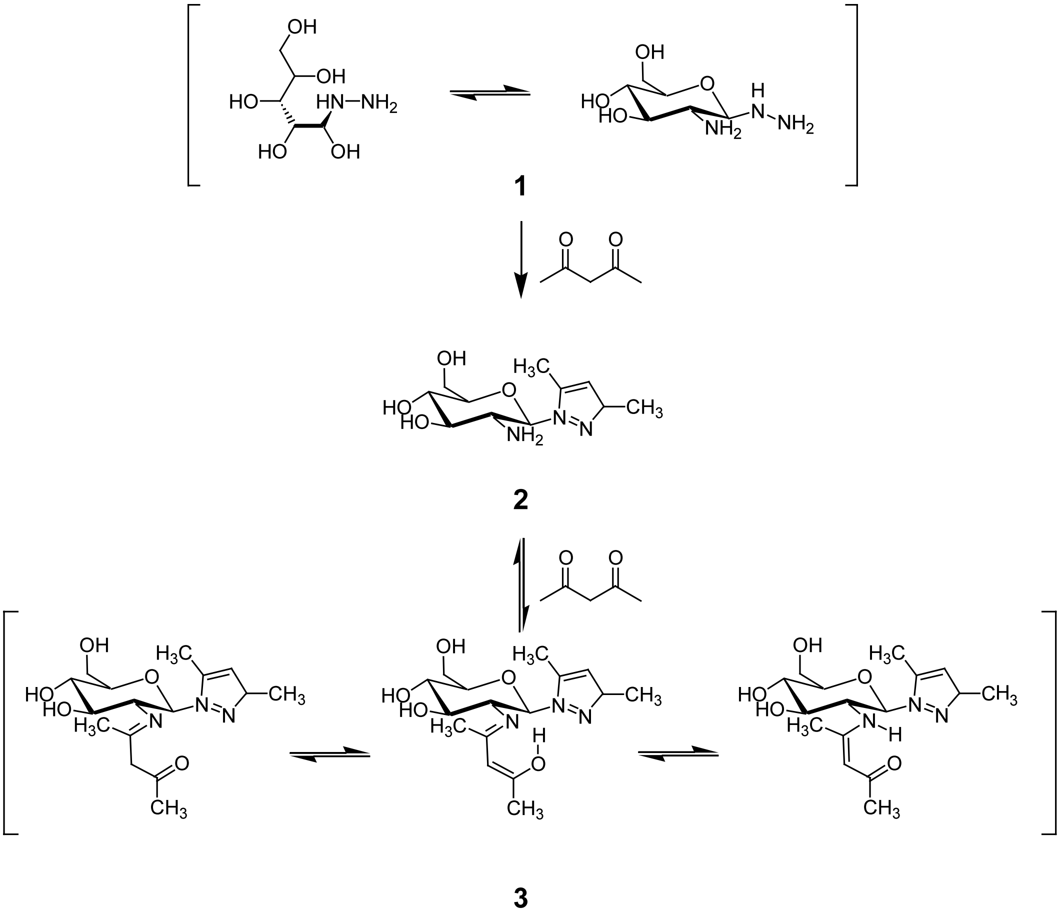
The major pyrazole isomer formed from glucosamine hydrazone is 1-(2-amino-2-deoxy-β-D-glucopyranosyl)-3,5-dimethyl-1*H*-pyrazole (**2**),[1] and further reaction with acetylacetone yields the ketoenamine 1-[2-deoxy-2-(4-oxo-2-pentenyl)amino)-β-D-glucopyranosyl)]-3,5-dimethyl-1*H*-pyrazole (**3**) (Fig. 1). Formation of **3** by addition of excess acetylacetone to **2** was monitored using reversed-phase HPLC with diode array detection. The retention times of compounds **2** and **3** were established using authentic samples prepared as described in

---

* Present address: Angiogenesis Research Laboratory, Dartmouth Medical School, Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon, NH 03756, USA. Tel.: +1-603-6530510; fax: +1-603-6500820

E-mail address: warren.kett@dartmouth.edu (W.C. Kett).

0008-6215/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0008-6215(03)00046-6



Scheme 1. The major products in the reaction of acetylacetone with glucosamine hydrazone, **1**. For clarity, only the β-pyranosyl form of the sugar ring is shown.

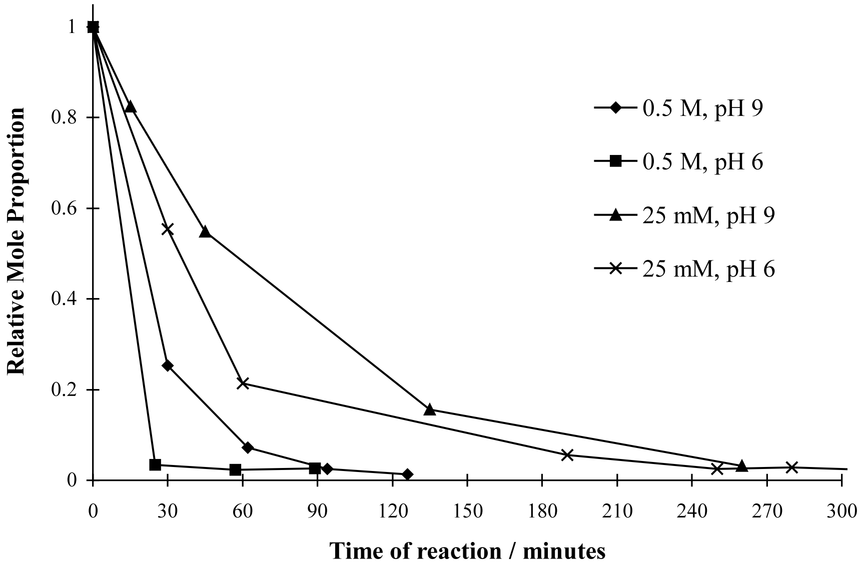


Fig. 1. Time course of the reaction in phosphate buffers at 22 °C of compound **2** with acetylacetone. There was a 25-fold molar excess of acetylacetone and the reaction was monitored by RP-HPLC with 1-β-D-glucopyranosyl-3,5-dimethyl-1*H*-pyrazole included as an internal standard.

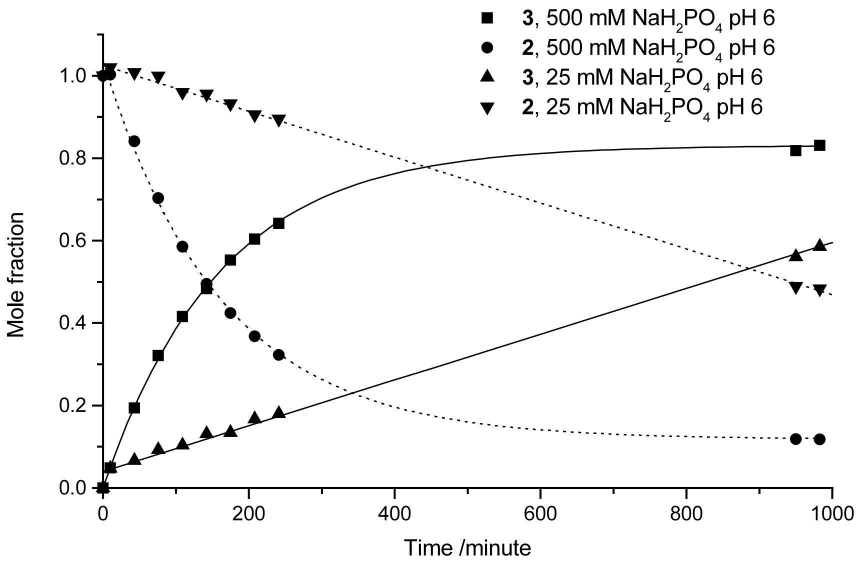


Fig. 2. Time course of the hydrolysis of compound **3** to compound **2** in Na_2HPO_4 buffer, pH 6 at 22 °C. Analysis was by RP-HPLC.

the experimental section. For quantitative studies, 1-β-D-glucopyranosyl-3,5-dimethyl-1*H*-pyrazole, which was available from previous studies,[1] was included as internal standard.

In unbuffered water, the rate of coupling was slow and less than 10% reacted in 3 h, but the presence of phosphate buffer increased the rate dramatically (Fig. 1). The disappearance of the free amine **2** was correlated with the appearance of the ketoenamine **3**.

The hydrolysis of **3**, after the removal of excess acetylacetone by evaporation, was followed in a similar fashion. At room temperature, hydrolysis was slow, and 16 h incubation in 0.5 M phosphate buffer at pH 6 effected hydrolysis of only 85% of the starting material (Fig. 2). Complete regeneration of **2** was achieved within 2 h at 70 °C by incubation with aqueous hydrazine (Fig. 3).

2.2. Enamines from chitooligosaccharides

The reaction of acetylacetone with oligosaccharides possessing multiple amino groups was of critical interest and most pertinent to glycan derivatisation. Pyrazole derivatives of chitooligosaccharides containing between two and six glucosamine subunits were used to study the efficiency of ketoenamine formation when multiple amino groups were present. Chitosan was hydrolysed, the products were converted to hydrazones and excess acetylacetone was added to enable reaction with the amino moieties as well as conversion to dimethylpyrazoles. The *per*ketoenamine of each chitooligosaccharide pyrazole was isolated from the reaction mixture using reversed-phase HPLC (Fig. 4) and used for further studies.

The chromatographic properties of partially substituted ketoenamines of the chitooligomer of DP 3 were determined by following the hydrolysis of the corresponding *per*ketoenamine by reversed-phase HPLC and online ESIMS (Fig. 5). It was then possible to search for conditions to achieve optimal yields. The equilibrium proportions of isomers were found to depend on the pH, but the maximum yield of the *per*ketoenamine, which occurred at pH 7, was only 90 ± 4% (Fig. 6), with significant amounts of the *bis*ketoenamine isomers present.

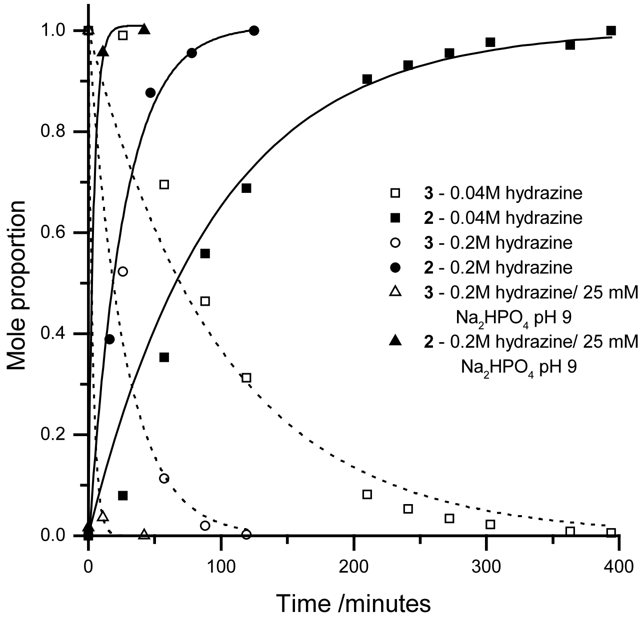


Fig. 3. Time course of the conversion of the ketoenamine, **3** (hollow symbols), to the amine, **2** (filled symbols), on treatment with aqueous hydrazine at 70 °C.

The extent of ketoenamine formation for the other oligomers at pH 7 was established by incubation for 16 h (Table 1). In each instance, the only significant products were the *per*ketoenamine derivative and those with a single free amino group. Confirmation of peak identity was achieved by LC–MS and peak areas were corrected for the expected differences in extinction coefficients, based on the assumption that each ketoenamine moiety made the same contribution to the total molar absorptivity. It is possible, for the oligomers of DP 5 and 6 that the yield of *per*ketoenamine was slightly overestimated, because the broad peak resulting from excess acetylacetone may have obscured minor components attributable to products bearing two or more free amino groups.

As it seemed unlikely that ketoenamines could be exploited for analysis, it was necessary to establish conditions to eliminate them. The *per*ketoenamine of the chitooligomer of DP 3 hydrolysed slowly in water (Fig. 5A), and after 3 days 40% of the *tris*ketoenamine was still present (Fig. 5B). Hydrolysis in 0.2 M triethanolamine–phosphate buffer at pH 7 was faster and equilibrium was established within 20 min, but whilst all the *tris*ketoenamine had hydrolysed, there was still an equilibrium mixture of *bis*ketoenamines and monoketoenamines (Fig. 5C and D). Thus, treatment with hydrazine would appear to be the more effective means to remove all ketoenamines before proceeding with glycan analysis.

3. Discussion

These results demonstrate that conversion of glycosylhydrazones to pyrazoles will be accompanied by significant side reactions if amino sugars are present. In many cases they occur naturally in the N-acetyl form, but during hydrazinolysis, extensive, although incomplete, deacetylation occurs.[8,10,11] Consequently, there are always likely to be exposed amino groups in routine samples.

Ketoenamines did not cause a problem in our earlier study[1] for reasons that would not apply to routine analytical procedures. In that work, the pyrazoles were prepared without the use of buffers, which have now been shown to catalyse enamine formation (Fig. 1). This is an example of the general acid catalysis of imine formation,[12,13] and a similar effect has been observed for the formation of enaminals from glycosylamines and malondialdehyde, for which the maximum rate occurred at pH 3–4 and yields exceeded 95%.[14] It is unlikely that in a general analytical procedure the presence of proton donors could be avoided. Most samples mixtures would contain amino acids and peptides, as well as residual buffer salts.

A second reason why enamine formation was unimportant previously is that workup involving extensive extraction and subsequent peracetylation probably resulted in hydrolysis of the small amounts of ketoenamine that had been formed.

The strategy of converting amino sugars into *per*ketoenamines came tantalisingly close to success. Although high yields were obtained (> 90%), the multiple products formed by the remainder is problematic. The incomplete conversion of oligosaccharides may be the result of steric interference, as has been suggested for the formation of Schiff's base derivatives of chitosan [[15] and references therein]. It may still prove possible to use the method with glycans, such as glycosaminoglycans, in which the amino sugars are not adjacent. The

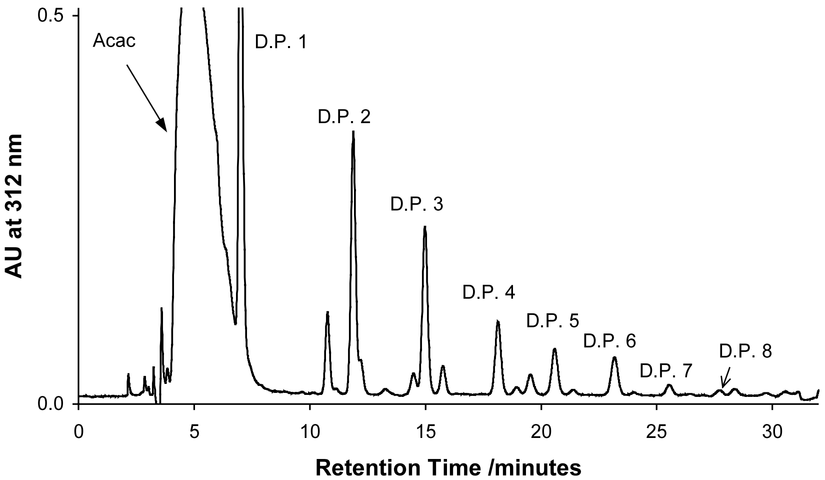


Fig. 4. RP-HPLC of the dimethylpyrazole derivatives of chitooligosaccharides prepared after partial acid hydrolysis of chitin. ESIMS indicated the major species were *per*ketoenamines. Unlabelled peaks were not identified. An Alltima C_{18} column, 250 × 4.6 mm, was used with a flow rate of 1 mL/min and a gradient comprised of water:acetonitrile (18:82 for 1 min, changed linearly to 72:28 at 26 min, and 50:50 at 30.5 min). Detection was at 312 nm.

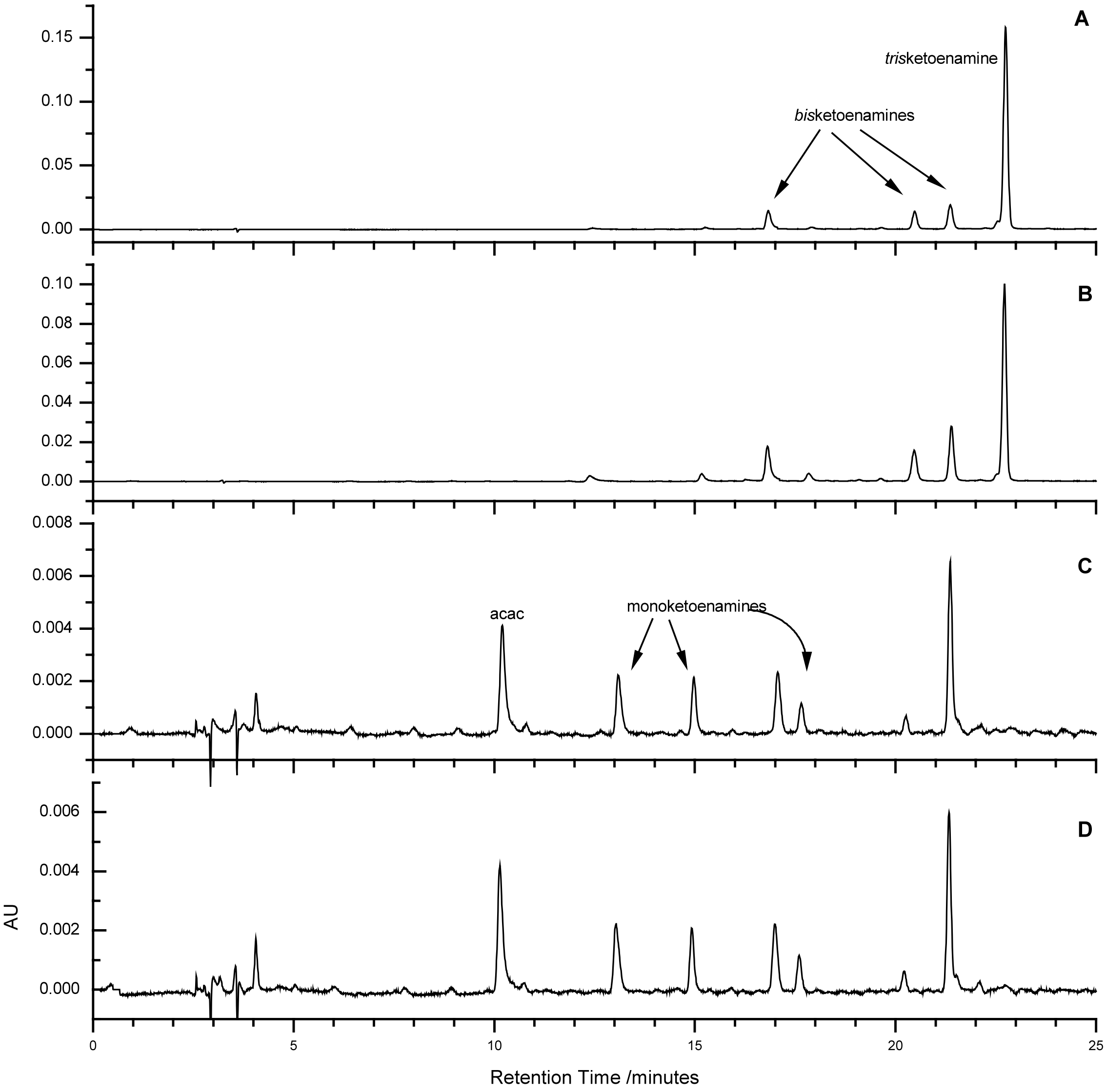


Fig. 5. RP-HPLC of the purified *tris*ketoenamine adduct of the 3,5-dimethylpyrazole derivative of chitotriose; (A) immediately after re-dissolution in water; (B) 3 days later; (C) 20 min after dissolution in 0.2 M triethanolamine acetate pH 7; (D) 4 h after C. Sample sizes were the same for each trace. An Alltima C_{18} column, 250 × 4.6 mm, was used with a flow rate of 1 mL/min and a gradient comprised of 95:5 25 mM NH_4PO_4 pH 5.8–acetonitrile (eluent A) and acetonitrile (eluent B). The eluent proportions were 97:3 A:B for 2 min, changed linearly to 68:32 A:B at 22 min, then to 40:60 A:B at 25 min and then maintained for 1 min. Detection was at 312 nm.

incomplete formation was disappointing because they would have been very useful for analysis because of their large extinction coefficients in the far UV region, favourable reversed-phase HPLC characteristics and efficient ionisation in an electrospray ionisation mass spectrometer.

The pH-dependent equilibrium distribution of partially substituted ketoenamines is most likely due to ionisation of the species involved in the equilibrium. Amino sugars have a pK_a of approx 7.5 (2-amino-2-deoxy-β-D-glucose, pK_a 7.66[16,17]), and the pK_a of acetylacetone is 9.2.[18] Small differences in the pK_a values of the partially substituted species could cause the observed behaviour. The rates of hydrolysis of several *N*-alkyl- and piperidino ketoenamines were reported to vary markedly and to depend on pH.[19] For some of the ketoenamines there was a distinct maximum or minimum in the rate of hydrolysis in the range from pH 2 to 7, whilst for others there was a simple linear relationship between rate and pH. The rate of hydrolysis of compound **3** apparent from Fig. 2 is similar to that for the ketoenamine formed from ammonia and acetylacetone and considerably slower than the *N*-alkyl ketoenamines.[19]

The slow and incomplete hydrolysis of ketoenamines derived from monosaccharides and *per*ketoenamines formed from oligosaccharides that was observed in this study contrasts with the ease of decomposition reported

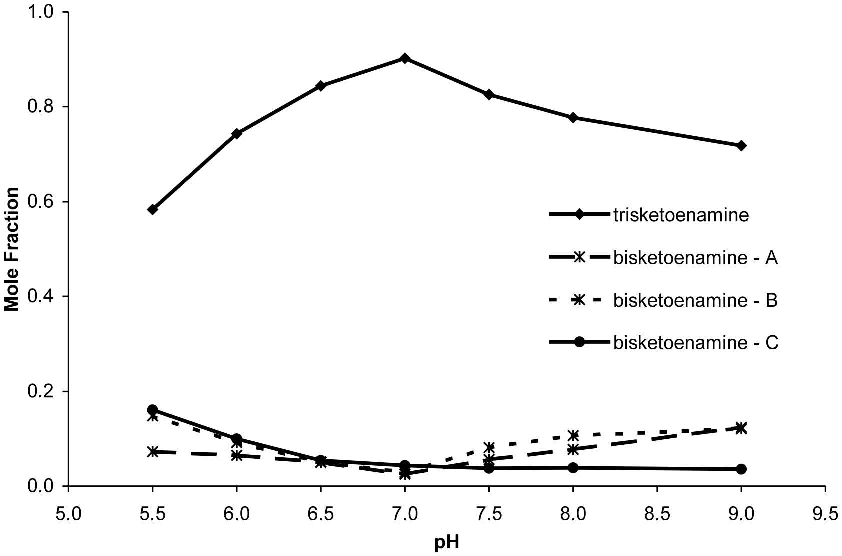


Fig. 6. Distribution of the ketoenamines of the 3,5-dimethylpyrazole derivative of chitotriose as a function of pH. Analysis was performed by RP-HPLC.

for the ketoenamine formed from acetylacetone and glucosamine,[8] but in the latter case, further reaction to form pyrroles and other Elson–Morgan products occurs. Our results suggest that it is this subsequent reaction which contributes to the apparent lability of the ketoenamines.

It was possible, however, to remove the ketoenamine moiety from compound **3** completely and conveniently using hydrazine. This is an example of the general use of ketoenamines as synthons for heterocycles[20,21] and generates further 3,5-dimethyl-1*H*-pyrazole as a byproduct. This is attractive for analytical applications, particularly as the excess of hydrazine could be removed by conversion to the volatile diazine by addition of acetone.

The condensation of β-functional reagents with mono-substituted hydrazines is a general strategy for the synthesis of five-membered heterocycles.[22] We have extended this approach to include the synthesis of 1-glycosylpyrazoles and 1-glycosylpyrazolones from saccharide hydrazones,[1,23] but the current study sounds a cautionary note for application to biologically active glycans if the glycan possesses latent amino groups. As this includes all N-linked glycans and most O-linked glycans of glycoproteins, the obvious ramification for analytical strategies is that extra steps and manipulations will be required to eliminate this side-reaction.

4. Experimental

4.1. General

All materials were obtained from commercial sources and used without further purification. 1-aldosyl 3,5-dimethyl-1*H*-pyrazoles were prepared as described previously.[1] NMR spectra were acquired at 27 °C on a Varian XL-400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C observation. Proton decoupling was used to assist in spectral assignment. Chemical shifts are reported downfield from Me_4Si, which was used as the internal reference. The HPLC system was comprised of two LC-10 pumps, SGU-104 solvent degassing unit, SPD-10MA photodiode array detector, 10Axl autosampler, manual Rheodyne injector (Rheodyne, Cotari, USA), and FRC-10 fraction collector. The pumps were configured for either binary high-pressure mixing. Data acquisition and instrument control was achieved using Shimadzu CLASS 5000 software. Electrospray-ionisation mass spectrometry (ESIMS) was performed with a Fisons VG Quattro spectrometer (VG Analytical, Manchester, England). Data acquisition and instrument control was achieved using Mass Lynx software (VG Analytical, Manchester, England). The Shimadzu HPLC was coupled with the mass spec-

Table 1
Yields of *per*ketoenamines of chitooligosaccharide 3,5-dimethylpyrazole derivatives obtained in aqueous buffer at pH 7[a]

DP	Mole percent of *per*ketoenamine (%)
2	94 ± 2
3	90 ± 4
4	94 ± 5
5	90 ± 5
6	90 ± 6

[a] Analysis was performed by RP-HPLC.

trometer for LC–MS experiments. A 10:1 split ratio between the detector effluent and the mass spectrometer inlet was achieved using a T connector.

4.2. Preparation of 1-[2-deoxy-2-(4-oxo-2-pentenyl)-amino)-β-D-glucopyranosyl)]-3,5-dimethyl-1*H*-pyrazole (3)

A mixture of isomeric dimethylpyrazole derivatives of glucosamine was prepared using previously described methods and contained mostly 1-(2-amino-2-deoxy-β-D-glucopyranosyl)-3,5-dimethyl-1*H*-pyrazole (**2**).[1] The crude gum (5 g), was dissolved in dry MeOH (40 mL), 1 mL of Et_3N was added, and the solution was refluxed for 2 h.[8] The solution was concentrated to about 10 mL in a rotary evaporator and allowed to cool. The resulting light brown crystals of **3** (1 g), which were found to contain traces of Et_3N, were filtered. A small amount of the solid was purified by RP-HPLC for NMR studies using a Jordi divinylbenzene column (100 × 10 mm) and a binary gradient system comprised of 90:10 water–MeCN (eluent A) and MeCN (eluent B). The eluent proportions were 15:85 A:B for 2 min, changed to 50:50 A:B at 17 min. The eluted species were detected at 220 and 312 nm; Positive-ion ESIMS/MS: m/z 340 $[M+H]^+$; ^1H NMR (Me_2SO-d_6): 11.16 (d, 1 H, N–H, $J_{2,NH}$ 10.8 Hz), 5.79 (s, 1 H, pyrazole H-4), 5.45 (d, 1 H, H-1, $J_{1,2}$ 9.3 Hz), 5.28 (d, 1 H, 3-OH, $J_{3,OH}$ 6.2 Hz), 5.11 (d, 1 H, 4-OH, $J_{4,OH}$ 5.2 Hz), 4.78 (s, 1 H, H-3′), 4.57 (dd, 1 H, 6-OH, $J_{6,OH}$ 5.7 Hz), 4.26 (ddd, 1 H, H-2, $J_{2,3}$ 9.7 Hz), 3.67 (m, 1 H, H-6, $J_{6,6'}$ 12.6 Hz), 3.4–3.5 (m, 3 H, H-3/H-5/H-6′, $J_{3,4}$ 9.2 Hz, $J_{5,6}$ 1.4 Hz), 3.22 (ddd, 1 H, H-4, $J_{4,5}$ 9.2 Hz), 2.17, 2.07, 1.86 and 1.79 (4 methyl groups); ^{13}C NMR (DMSO-d_6): 193.1 (C-4′), 163.4 (C-2′), 146.9 and 140.6 (pyrazole C-2 and C-4), 105.3 (pyrazole C-3), 95.1 (C-3′), 83.0 (C-1), 78.6 (C-5), 76.3 (C-3), 70.3 (C-4), 60.8 (C-6), 57.7 (C-2), 28.5 (C-5′), 18.7 (C-1′), 13.3 and 10.1 (pyrazole methyl groups). The ^1H and ^{13}C NMR spectra were similar to those reported for the ketoenamines formed from glucosamine[3] and mannosamine,[4] apart from the signals arising from the pyrazole moiety. The diagnostic resonance was the ^1H doublet for the anomeric proton that occurs 0.1 ppm further upfield than that for the anomeric proton in the precursor amine, 1-(2-amino-2-deoxy-β-D-glucopyranosyl)-3,5-dimethyl-1*H*-pyrazole (**2**).

4.3. HPLC analysis of the reaction between acetylacetone and 1-(2-amino-2-deoxy-β-D-glucopyranosyl)-3,5-dimethylpyrazole

The 3,5-dimethylpyrazole derivatives of glucosamine and glucose (for use as an internal standard) were prepared as described for the 1-aldosyl-3,5-dimethylpyrazoles.[1] The resulting gums were used to prepare a stock solution containing approximately 10 mg/mL of each derivative. A 50-μL aliquot (approx 2 μmol) was placed in an autosampler vial with 0.3 mL of buffer and 2,4-pentanedione (5 μL, 50 μmol). The vial was sealed and the solution analysed at intervals by HPLC using an Alltima C-18, 250 × 4.6 mm column (Alltech Associates, Sydney, Australia). The column was eluted using a binary gradient comprised of 95:5 25 mM ammonium phosphate pH 5.8–acetonitrile (eluent A) and acetonitrile (eluent B). The eluent proportions were 95:5 A:B for 5 min, changed linearly to 71:29 A:B at 16 min, then to 40:60 A:B at 19 min, where it remained for 2 min. The eluted species were detected at 220 and 312 nm.

4.4. Hydrolysis of 1-[2-(deoxy-2-(4-oxo-2-pentenyl)-amino)-β-D-glucopyranosyl)]-3,5-dimethylpyrazole (3)

The 1-[2-(deoxy-2-(4-oxo-2-pentenyl)amino)-β-D-glucopyranosyl)]-3,5-dimethylpyrazole (**3**), prepared as described above, was dissolved in 100 mL of MeOH and stored at 4 °C. An aliquot of this stock solution (50 μL, approx 2 μmol) was placed in a vial with 500 μL of buffer and 50 μL of glucosyl-3,5-dimethylpyrazole solution (10 mg/mL). If desired, an aliquot of hydrazine hydrate (2–5 μL, 40–100 μmol) was also added. The progress of the hydrolysis was monitored by HPLC as described above.

4.5. Preparation of ketoenamine adducts of chitooligosaccharide 3,5-dimethylpyrazole derivatives

Concentrated HCl (30 mL) was added to chitosan (200 mg) and boiled with vigorous stirring for 2 h. The acid was removed by rotary evaporation, and the sample was dissolved in water (20 mL). Hydrazine hydrate (5 mL) was added, followed by HOAc, added dropwise until the sample redissolved. After incubation at room temperature overnight, the hydrazine was removed in a rotary evaporator. The residue was suspended in water (40 mL), acetylacetone was added (10 mL) and the mixture was left to stand overnight. The resulting suspension was filtered, and the filtrate was washed with $CHCl_3$ (3 × 25 mL) to remove 3,5-dimethylpyrazole. To maximise the yield of ketoenamine adducts, excess acetylacetone (2 mL) was added, and the solution was concentrated to near dryness before being redissolved in 25% MeOH (10 mL) and passed through a 0.45-μm filter. The sample was analysed by RP-HPLC–MS using 18:82 water–acetonitrile for 1 min, changed linearly to 72:28 at 26 min, and 50:50 at 30.5 min. The eluted compounds were detected at 220 and 312 nm. The mass spectrometer was scanned over the range 80–2000 amu and the skimmer cone voltage was 35 V.

4.6. Purification of ketoenamine adducts of chitooligosaccharide pyrazole derivatives

A total of 1 mL of the crude mixture, prepared as described above, was fractionated by repetitive injection with automatic fraction collection. The conditions were as described above. The fractions were rotary evaporated to dryness and repurified. The initial proportion of acetonitrile was varied to suit each fraction. For example, the solvent gradient employed for the tetramer was 80:20 water–acetonitrile initially for 1 min, changed linearly to 67:33 at 27 min. The fractions were evaporated to dryness (centrifugal evaporator) and redissolved in water (200–500 μL).

4.7. Monitoring the formation of ketoenamine adducts of chitooligosaccharides

An aliquot of a purified chitooligosaccharide derivative, prepared as described above, was placed into an autosampler vial, and either 0.2 M triethanolamine phosphate buffer or water was added. The mixture was incubated at room temperature and 20 μL samples were analysed by RP-HPLC at regular intervals. The oligomers of DP 2–3 were detected at 220 and 312 nm and eluted with a gradient formed from 25 mM NH_4PO_4 pH 5.8–acetonitrile (eluent A) and acetonitrile (eluent B). The eluent proportions were 97:3 A:B for 2 min, changed linearly to 68:32 A:B at 22 min, then to 40:60 A:B at 25 min and then maintained for 1 min. For ESIMS, and for studying oligomers of DP 4–6, the eluents were 95:5 16 mM NH_4OAc pH 5.8–acetonitrile (eluent A) and acetonitrile (eluent B), and the detection wavelength was changed to 312 nm. The gradient was 92:8 A:B for 2 min, changed linearly to 62:38 A:B at 22 min, and to 40:60 A:B at 25 min where it was maintained for 1 min. The identities of all the ketoenamine adducts were corroborated by online ESIMS. In-source collision-induced fragmentation data was acquired using a skimmer cone voltage of 75 V.

Acknowledgements

I express my appreciation to Michael Batley and John Redmond (Macquarie University) for helpful discussions throughout the course of this study and the preparation of the manuscript.

References

1. Kett, W. C.; Batley, M.; Redmond, J. W. *Carbohydr. Res.* **1997**, *299*, 129–141.
2. Hase, S. Pre- and post-column detection oriented derivatisation techniques in HPLC. In *Carbohydrate Analysis*: *High Performance Liquid Chromatography and Capillary Electrophoresis*; El Rassi, Z., Ed.; Elsevier: Amsterdam, 1995; pp 555–575.
3. Honda, S. *J. Chromatogr., Sect. A* **1996**, *720*, 337–353.
4. Patel, T.; Bruce, J.; Merry, A.; Bigge, J.; Wormald, M.; Jaques, A.; Parekh, R. *Biochemistry* **1993**, *32*, 679–693.
5. Patel, T.; Parekh, R. B. *Methods Enzymol.* **1993**, *230*, 57–66.
6. Cooper, C. A.; Packer, N. H.; Redmond, J. W. *Glycoconjugate J.* **1993**, *11*, 163–167.
7. Garcia Gonzalez, F.; Gomez Sanchez, A. *Adv. Carbohydr. Chem.* **1965**, *20*, 303–355.
8. Gomez Sanchez, A.; Gomez Guillen, M.; Scheidegger, U. *Carbohydr. Res.* **1967**, *3*, 486–501.
9. Bhacca, N. S.; Ludoweig, J. J. *Carbohydr. Res.* **1969**, *11*, 432–434.
10. Carter, S. R.; Williams, J. M.; Clamp, J. R. *Carbohydr. Res.* **1990**, *205*, 181–190.
11. Amano, J.; Nishimura, R.; Mochizuki, M.; Kobata, A. *J. Biol. Chem.* **1988**, *263*, 1157–1165.
12. Sayer, J. M.; Conlon, P. *J. Am. Chem. Soc.* **1980**, *102*, 3592–4002.
13. Cordes, E. H.; Jencks, W. P. *J. Chem. Soc.* **1963**, *85*, 2843–2848.
14. Gomez Sanchez, A.; Maya, I.; Hermosin, I. *Carbohydr. Res.* **1992**, *229*, 307–322.
15. Domszy, J. G.; Roberts, G. A. F. *Int. J. Biol. Macromol.* **1985**, *7*, 45–48.
16. Neuberger, A.; Fletcher, A. P. *J. Chem. Soc., Sect. B* **1969**, 178–181.
17. Blasko, A.; Bunton, C. A.; Bunel, S.; Ibarra, C.; Moraga, E. *Carbohydr. Res.* **1997**, *298*, 163–172.
18. Bruice, P. Y. *J. Am. Chem. Soc.* **1990**, *112*, 7361–7368.
19. Dixon, K.; Greenhill, J. V. *J. Chem. Soc., Perkin Trans. 2* **1974**, 164–168.
20. Kucklander, U. Enaminones as synthones. In *The Chemistry of Enamines*; Rappoport, Z., Ed.; John Wiley and Sons: London, 1994; Vol. 1, Chapter 10, pp 523–636.
21. Lue, P.; Greenhill, J. V. *Adv. Heterocycl. Chem.* **1996**, *67*, 207–343.
22. Elguero, J. Pyrazoles and their benzo derviatives. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Pergamon Press: Oxford, 1984; Vol. 5, pp 167–303.
23. Kett, W. C.; Batley, M.; Redmond, J. W. *Carbohydr. Res.* **2000**, *329*, 169–177.